

second imaging subsystem best focused conjugate image for second target object (second image of second target) 298. For the implementation depicted in Figure 23, the first optical axis 286 and the second optical axis 296 are orthogonal with respect to one another.

With respect to the example shown in Figure 23, the second target object 282 and the first target object 292 occupy the same position with respect to the direction of the first optical axis 286. However, with respect to the direction of the second optical axis 296, the first target object is closer to the second imaging sub-system 276 than is the second target object. As shown in Figure 23, a first lateral shift 300 exists between the first image of second target 288 and the first image of first target 290 along the surface of the first detector 274 since the second target object 282 and the first target object 292 occupy the same position with respect to the first optical axis 286 and not with respect to the orthogonal second optical axis 296. As shown in Figure 23, the second detector 278 is located with respect to the best focus position for the first target object 292. Since the second target object 282 is farther away than the first target object 292 from the second imaging sub-system 276, the second image of second target 298 is located a focus shift for second imaging sub-system image 302 from the second detector 278.

Due to the orientation between the first imaging sub-system 272 and the second imaging sub-system 276, the focus shift for second imaging sub-system image 302 is proportional to the first lateral shift 300. In some implementations, the processor 280 is communicatively linked by communication links 503 to the first detector 274 and/or the second detector 278 to determine lateral displacements such as the first lateral shift 300. The processor 280 can further be communicatively linked by the communication links 503 to the first detector 274, second detector 278, the first imaging sub-system 272, and/or the second imaging sub-system 276 to either adjust the position of the first detector or the second detector, or adjust optical characteristics of the first imaging sub-system or the second imaging sub-system based upon determined displacements to correct for focus shifts such as the focus shift for second imaging sub-system image 302. For instance, as illustrated in Figure 23, the processor 280 could determine that the first lateral shift 300 occurred as the first focused light 284 moved from first image of first target 290 to the first

image of second target 288 as the flow cell cavity 268 first contained the first target object 292 and then contained the second target object 282. As a consequence of this determination, the processor 280 would instruct the second imaging sub-system 276 to move the second image of second target 298 to the second detector 278 based upon the first lateral shift 300. Alternatively, the processor 280 would instruct the second detector 278 to move to the current position of the second image of second target 298 as shown in Figure 23.

The relationship between lateral shifts, such as the first lateral shift 300, and focus shifts, such as the focus shift for second imaging sub-system image 302, is further elaborated by use of Figure 24 showing a representative example of the first detector 274 having a plurality of a first detector picture element 304, each being approximately 10 microns in size, in this representative example, arranged in rows and columns. The first image of first target 290 of the first target object 292 is shown as an exemplary cell having a cytoplasm and cell nucleus. Since the first target object 292 is in focus at the second image of second target 298, the lateral position along the x-axis of the centroid of first image of second target 308 of its first image of first target 290 defines the location of the ideal focal plane for a orthogonal axis 306 along the y-axis on the first detector 274. The first image of second target 288 of the second target object 282 is also shown as a cell having a cytoplasm and cell nucleus. Since the second target object 282 is not positioned at the ideal focal plane for the second detector 278, the second target object is imaged off-axis on the first detector 274 and a centroid of first image of first target 310 of its first image of second target 288 exhibits the first lateral shift 300 in position from the on-axis of the first image of first target 290 of the first target object 292. The amount of lateral shift between images at the first detector 274 is determined by the separation of objects, such as the first target object 292 and the second target object 282, and the lateral magnification of the first imaging sub-system 272. The amount of defocus between images at the second detector 278 is determined by the separation of the objects and the magnification along the second optical axis 296 or the longitudinal magnification of the second imaging sub-system

276. It is to be noted that the longitudinal magnification of the optical system, in these examples, is equal to the square of the lateral magnification.

In a typical implementation, magnification of optical systems such as the first imaging sub-system 272 in the second imaging sub-system 276 is 10X, with a pixel size on the detectors, such as the first detector 274 and the second detector 278, being 10 microns. In Figure 24, a five pixel or 50 micron positive lateral shift along the x-axis on the first detector 274 for the centroid of first image of first target 310 is shown. In this representative example, given a 10X magnification, a 50 micron positive lateral shift along the x-axis translates into a five micron shift along the optic axis, such as the second optical axis 296, away from the second imaging sub-system 276. In order to correct focus, the second detector 278 should be moved approximately 500 microns (five micron error x lateral magnification x lateral magnification) toward the second imaging sub-system 276. In these implementations, centroids, such as the centroid of first image of second target 308 and the centroid of first image of first target 310, are calculated using conventional methods. Some implementations keep a running average of multiple cell centroid locations to normalize any inconsistencies in cell shape before instructing an electromechanical system associated with either the detectors, such as the first detector 274 and the second detector 278, or optical subsystems, such as the first imaging sub-system 272 and the second imaging sub-system 276, for correction of focus error.

In general, information from each of the imaging sub-systems, such as the first imaging sub-system 272 in the second imaging sub-system 276, may be used to correct focus of one another. The target objects, such as the second target object 282, the first target object 292, and other target objects including other types of cells, do not need to lie along one of the optical axes of the imaging sub-systems, such as the first optical axis 286 and the second optical axis 296 in order to determine centroids of the target objects and to ascertain lateral shift. Implementations are used with magnification at various levels as long as corresponding lateral displacements are properly translated into focus error and subsequently proper correction is implemented. Many sorts of elements conventionally known can be translated in order to correct for focus error; therefore, the representative